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TITLE: Rapid in Vivo Validation of Tumor Suppressor Gene Function in Prostate Cancer Progression

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14. ABSTRACT

We have established a powerful system to interrogate CRISPR efficiency in a standard 3 day cell culture transfection system. Our system works exactly as anticipated and we were able to identify potent sgRNAs that target the tumor suppressors p53 and Rb. The lentiviral constructs that we designed and cloned efficiently disrupted a heterologous gene construct containing an mCherry cDNA fused to the sgRNA target. As predicted, we observed variable efficiencies between sgRNAs. Our assay facilitated the rapid identification of the best sgRNA sequences and accelerated our ability to move to the in vivo studies proposed in Aim2.

Our goal was to use CRISPR/Cas lentiviral transduction of the adult prostate to inactivate p53 or Rb. We timed to recapitulate the effects of conditional floxed alleles of p53 and Rb and to initiate prostate cancer in the mouse after injection of lentiviral particles expressing CRISPR/Cas components and Cre recombinase. Our initial efforts were thwarted by the negative impact that the relatively large size of our lentiviral construct had on our ability to generate high-titer lentivirus needed for the infection. We circumvented this by re-designing the lentiviral construct to delete unnecessary viral elements and utilize alternative promoter sequences that were smaller to overall reduce the size of the lentiviral genome to be packaged. This effort allowed us to generate 10 to 50 fold higher viral titers. We have used these viral preps to infect the adult prostate of mice. Currently, we are aging mice to determine the effects of the procedure. At this time the animals are 20 weeks post infection and, based on the published latency of the model, we anticipate the onset of tumors between 35 and 60 weeks post infection.

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Introduction

Identifying the functional significance that prostate cancer-associated mutations have on the progression of prostate cancer is critical to distinguish lethal forms of the disease to those that are indolent. Our goal was to establish CRISPR/Cas9-based methods to inactivate genes of interest within a genetically engineered mouse model of prostate cancer. Validation of our approach establishes a strong experimental platform to systematically interrogate genes that are significantly mutated in human prostate cancer.

Keywords

CRISPR/Cas9, Genetically engineered mouse (GEM) model, short guide RNA (sgRNA)

Accomplishments

Objectives

Our overall objective is to create an *in vivo* system to assess the impact of gene loss on the progression and pathophysiology of prostate cancer without the need to create or breed new genetic alleles. We will accomplish this goal with the following specific aims:

Aim1: Develop *in vitro* methods to identify effective guide RNAs to quantify CRISPR/Cas9 efficiency

We have established a powerful system to interrogate CRISPR efficiency in a standard 3 day cell culture transfection system. Our system works exactly as anticipated and we were able to identify potent sgRNAs that target the tumor suppressors p53 and Rb. The lentiviral constructs that we designed and cloned efficiently disrupted a heterologous gene construct containing an mCherry cDNA fused to the sgRNA target. As predicted, we observed variable efficiencies between sgRNAs. Our assay facilitated the rapid identification of the best sgRNA sequences and accelerated our ability to move to the in vivo studies proposed in Aim2.

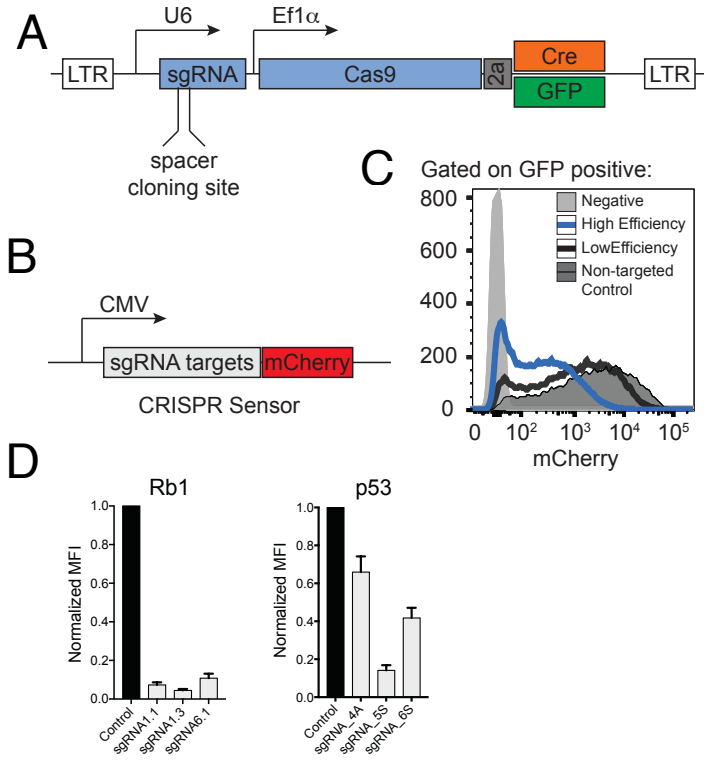

Figure 1. **A.** Schematic of CRISPRv2Cre and CRISPRv2GFP. Each vector expresses sgRNA, Cas9, and either Cre or GFP. **B.** Schematic of the Sensor construct expressing mCherry with N'-terminal sgRNA target sites cloned in frame. **C.** Flow cytometry analysis of 3 representative constructs that demonstrate high, low, and off-target efficiencies to disrupt mCherry expression. 293 cells are transfected with the CRISPR Sensor construct harboring multiple sgRNA target sequences and individual CRISPRv2GFP vectors with a single sgRNA. Flow data is gated on GFP positive cells and assessment of mCherry expression shown. The leftward shift toward mCherry negativity indicates effective disruption of the sensor. **D.** Identification of effective sgRNAs targeting Rb1 and p53.

Aim2: Validate methods to target and functionally inactivate tumor suppressor genes *in vivo*

Our goal was to use CRISPR/Cas lentiviral transduction of the adult prostate to inactivate p53 or Rb. We aimed to recapitulate the effects of conditional floxed alleles of p53 and Rb and to initiate prostate cancer in the mouse after injection of lentiviral particles expressing CRISPR/Cas components and Cre recombinase. Our initial efforts were thwarted by the negative impact that the relatively large size of our lentiviral construct had on our ability to generate high-titer lentivirus needed for the infection. We circumvented this by re-designing the lentiviral construct to delete unnecessary viral elements and utilize alternative promoter sequences that were smaller to overall reduce the size of the lentiviral genome to be packaged. This CRISPRv2Cre vector allowed us to generate 10 to 50 fold higher viral titers than our original vector CRISPRv1Cre as well as an independently published pSecc vector. We have used these viral preps to infect the adult prostate of mice. Currently, we are aging mice to determine the effects of the procedure. We anticipate the onset of tumors between 35 and 60 weeks post infection. A few animals have recently developed prostate tumors that we are in the process of characterizing.

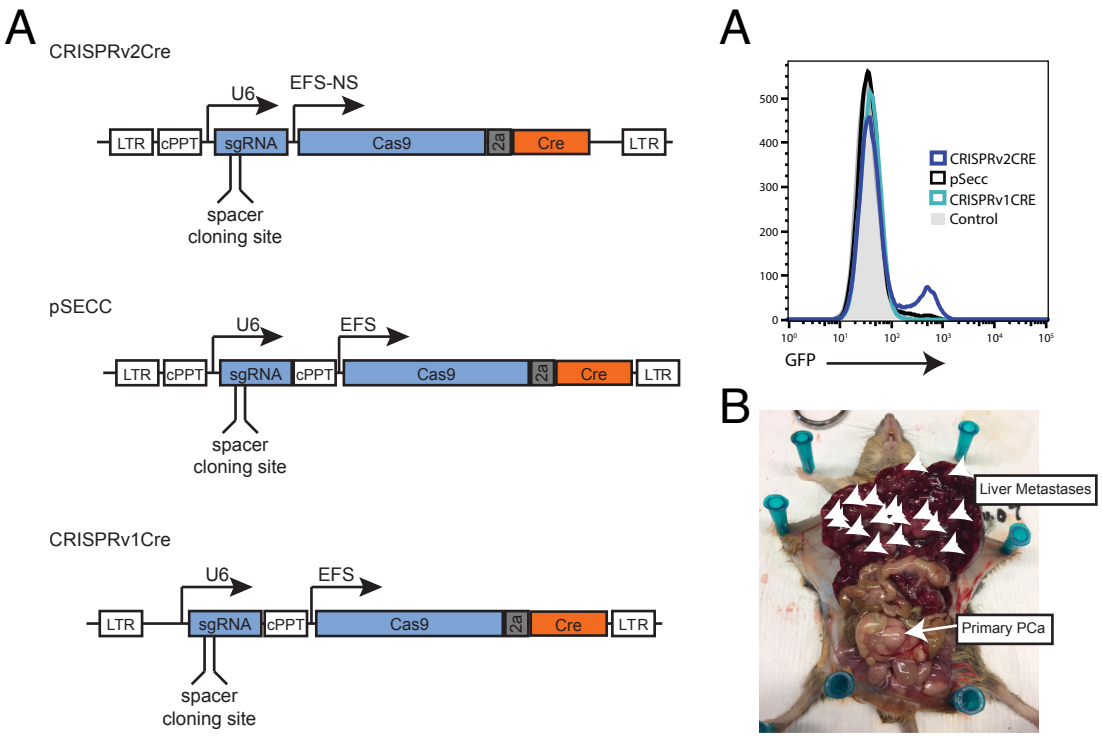

Figure 2. A. Schematic of the differences between our original CRISPRv1Cre, recently published pSecc, and our revised CRISPRv2Cre. Note changes in the promoter driving Cas9 expression and placement of the central polypurine tract (cPPT). Additionally note the duplication of the cPPT in the pSecc vector. **B.** Histogram detecting Cre-dependent GFP expression in cells used to titter cre-expressing lentivirus. Note the significant increase in viral titter of CRISPRv2Cre over both pSecc and CRISPRv1Cre. **C.** Representative p53flox/flox mouse infected with Rb targeting CRISPRv2Cre lentivirus. Note primary tumor mass (PCa) and numerous liver metastases (arrowheads).

Impact
Our method has the potential for great impact on the rapid validation of tumor suppressor gene activity in prostate cancer. Additionally, the newly designed lentiviral vectors will facilitate the addition of CRISPR-based approaches to other tumor models that rely on viral delivery of Cre recombinase.

Changes/problems: Problems with lentiviral production was discussed above. The issues were circumvented.

Products: New lentiviral vectors will be made freely available upon initial publication and will be distributed through Addgene.

Participants and other collaborating organizations: N/A

Special reporting requirements: None

Appendices: None